# ELECTRON MICROGRAPHS FROM CONCENTRATED SOLUTIONS OF THE TOBACCO MOSAIC VIRUS PROTEIN

by

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Optical and X-ray diffraction methods have provided much indirect evidence bearing on the way tobacco-mosaic-virus protein molecules are associated to cause the anisotropy of its solutions and its para-crystallinity as a solid. More direct observation of separate particles [1, 2] and of groupings of a few of them became possible with the development of the electron microscope, but the usual procedures for making microscopic preparations do not show the molecular arrangement in solutions or in solids. Such problems of particle arrangement can now be approached with the help of shadow replicas and of freeze-drying. Preliminary results illustrating their application are described in this paper.

Present knowledge of the particle arrangement in tobacco-mosaic-virus protein solutions and in the gels that form at high virus concentrations is largely due to BERNAL and FANKUCHEN [3] and has been summarized by them. BAWDEN and PIRIE [4] early pointed out that purified solutions containing more than 2 to 4 percent of this virus spontaneously separate into two layers on standing in the cold. The resulting upper portion is an opalescent liquid which is isotropic unless disturbed. The permanent sheen of the bottom component, however, indicates that its molecular particles are not haphazardly arranged. These bottom layers have physical and optical properties that depend on the virus concentration, and above about 35 percent virus they have the consistency of a gel. BERNAL and FANKUCHEN have recorded long X-ray reflections which measure the separation of regularly spaced particles in the bottom component; the decrease of these separations with water content is such as to indicate there is no abrupt change in phase with increase in virus concentration.

Work was undertaken to see if the particle arrangement in these concentrated solutions and gels could be photographed after quick-freezing [5] and drying from this frozen state and by using replicas to register the molecular configurations [6] on the surfaces of dried layers too thick for direct observation. As the photographs show, this can be done; but the extended study of the tobacco-mosaic-virus protein this makes possible will require much time and effort to complete.

The virus protein used in these experiments was grown in Turkish tobacco plants and purified by differential ultracentrifugation. It was dispersed and diluted with distilled water since good photographs can be made from frozen-dried samples only if little salt is present.

All electron microscopy was done with an RCA type EMU instrument operated with an objective aperture about 25 microns in diameter. Before microscopy, preparations were shadowed [7, 8] in an RCA type EMV evaporator by the deposition of an average thickness of ca. 8A of metallic gold, the angle of evaporation being such

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that shadows were five times the heights of the objects causing them. Air-dried preparations were made on collodion-covered screens ; some of the frozen-dried preparations were made directly on the wire grids without supporting membrane of any sort. Three kinds of preparations were examined : 1° air-drie ddilute solutions ; 2° air-dried concentrated solutions ; and 3° frozen-dried concentrated solutions. Air-dried dilute solutions were studied by depositing a microdrop upon a collodion-covered screen, allowing it to remain for a couple of minutes, then withdrawing as much as possible, and permitting the residue to dry in the air. Preparations from more concentrated solutions could not be made in this way because they were too thick for satisfactory microscopy. Micrographs of the molecular arrangement on the surface of such thick preparations of air-dried virus [2], could, however, be made with the help of shadowed replicas. Such replicas of dried, dilute, tobacco mosaic virus preparations have already been published [9]. Replicas have now been prepared in a similar fashion by drying concentrated material on a clean microscope slide, and shadowing the slide and virus with gold, which was then covered with collodion and stripped from the glass. This stripped film floating on water was cut with scissors, picked up on specimen screens, and after drying was ready for microscopy. Frozen-dried preparations [3], were made as previously described, aluminum instead of glass slides being used. When substrates were employed, collodion-covered screens were mounted on the metallic slides in the customary fashion. Preparations were made by chilling such a screen-covered slide on a block of metal cooled with dry ice, then adding and instantly withdrawing a droplet of the solution in question. When this was properly done, just enough of the solution remained frozen to the screen to give a finished preparation of the correct thickness for electron microscopy. The slide, with its frozen preparations, resting on its block of cold metal was quickly transferred to a suitable insulated position under the bell jar of the evaporator and a vacuum drawn. Under these circumstances, water sublimed from the unmelted preparations. When they were dry and the vacuum was adequate, they were shadowed and examined in the microscope. Experience has indicated that when a sufficient organization exists within a concentrated solution of fibrous molecules, electron microscopic preparations can be made without a supporting membrane. The tobacco mosaic virus protein is a material of this sort. Unsupported preparations were made in essentially the same manner by placing empty screens on chilled aluminum slides and depositing and then withdrawing a droplet of solution in the usual way. Only a limited use has been made of them because their depth, as established by the thickness of the metallic grid, has exceeded the focal depth of the microscope, with the result that only small parts of a field have been in best focus. Nevertheless, they have offered a most useful way of eliminating effects that could conceivably be attributed to the presence of a substrate.

Previous shadowed micrographs have shown tobacco mosaic rods individually and in small clusters distributed over the face of the preparation. Similar fields appear in the background of Figures 3, 4, and 5 which have been made from frozen-dried samples. Photographs of replicas taken from dilute solutions after drying on glass have been similar except that in them the fine structure of the collodion substrate has scarcely been perceptible. As concentration of solution was increased the rods became more numerous and areas of parallel arrangement became noticeable. Finally this parallelism became predominant and yielded micrographs of which Figure 1 is typical. As this picture indicates the fields from concentrated tobacco mosaic virus

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solutions have consisted of sheaves of roughly parallel rods which curve about, abut one another along remarkably straight lines and sometimes, as in the center of Figure 1, overgrow and partly enmesh one another. The curving sheaves are probably a pattern for the kind of particle arrangement that causes spiraling and alternating polarization in narrow tubes, as well as the tactoids that BERNAL and FANKUCHEN have described as being the result of remixing the organized bottom and unorganized top layers of a separated solution. None of the photographs yet made has shown a discrete positive tactoidal lens, but the mesh of Figure 5, from a frozen-dried preparation, can be interpreted as a negative tactoid.

In these tobacco mosaic photographs, the alignment may be very accurate for a few rods, as in parts of Figure 2, but extensive areas of great regularity have not yet been observed and order has not been seen to extend to the third dimension. Sometimes the rods have been closely packed and in contact, in other photographs the interval between centers has been at least 200A. Perhaps these differences in spacing are the consequence of interstitial water that undoubtedly was present before dehydration. More information about this can be obtained from the study of frozen-dried gels and of the so-called crystals that are obtained by acid precipitation and salting-out.

The most striking characteristic of the frozen-dried preparations has been the existence of sheets or tissues composed of aligned rods. A portion of such an organized sheet overlying a collodion film strewn with individual virus particles is shown in Figure 3. These sheets have been fragile and have broken up very readily under the electrons of the microscope. They would be seen covering extensive areas when a preparation was first put into the microscope, but they have immediately started to disintegrate, and it has been possible to photograph only the fragments that remained after this break-up had ended and the accompanying movement and drift within the preparation had ceased. The two-dimensional nature of the sheets is strongly suggestive of surface films such as might conceivably cover a drop of tobacco mosaic protein solution. When one considers, however, the way the frozen-dried preparations have been made and the fact that there often were successive layers of sheets, it is hard to avoid the conclusion that the sheets existed in the body of the solution. Their nature will undoubtedly become clearer through further work.

These tobacco mosaic sheets not only look like sheets of connective tissue and other substances composed of elongated molecules but they break up in the same fashion, forming shreds that roll up into bundles such as those shown in Figure 4. Such phenomena are especially interesting to study in the tobacco mosaic protein because its molecules are big enough to be easily visible.

These preliminary experiments are now being extended to the study of particle arrangement in tobacco mosaic gels and crystals. Results will be described in a later publication.

## SUMMARY

Shadowed metal replicas and freeze-drying can be used to make preparations whose electron micrographs show the molecular particle arrangement in concentrated solutions of the tobacco mosaic virus protein and in films dried from such solutions. Preliminary pictures of such preparations are reproduced showing continuous two-dimensional sheets and other regularities in particle arrangement.

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## RÉSUMÉ

L'utilization de préparations obtenues à l'aide de projection métallique et de la dissication à l'état congelé permet d'obtenir des micrographies électroniques montrant l'arrangement des particules moléculaires dans des solutions concentrées du virus de la mosaïque du tabac. Des photographies de telles préparations montrent une certaine organisation dans les feuilles à deux dimensions obtenues.

## ZUSAMMENFASSUNG

Schattierte Metallreproduktionen und Gefriertrocknung können angewandt werden zur Darstellung von Präparaten, deren Elektronmikrographien die molekulare Anordnung in konzentrierten Lösungen des Tabaksmosaikvirus-Proteins und in getrockneten Filmen aus solchen Lösungen zeigen. Vorläufige Bilder von solchen Präparaten, welche kontinuierliche zwei-dimensionale Blätter und andere Regelmässigkeiten in der Anordnung der Partikeln zeigen, werden reproduziert.


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